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Antioxidative Sesquiterpenes from *Artemisia iwayomogi*

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Artemisia iwayomogi, a member of the Compositae, is a perennial herb easily found in Korea and considerable attention has been paid to this medicine because the herbal plant is believed to have a chemopreventive potential.[1] Methanol extracts of *A. iwayomogi* inhibited nitric oxide production of lipopolysaccharide-activated macrophages[2] and two sesquiterpenes from *A. iwayomogi* were shown to inhibit the expression of inducible nitric oxide synthetase (iNOS).[3] In another study, methanol extracts of *A. iwayomogi* displayed scavenging activity of peroxynitrite ($ONOO^-$), a potent cytotoxic oxidant formed by the reaction between nitric oxide (NO) and superoxide radical (O^{2-}).[4] An aqueous extract of *A. iwayomogi* prevented the immediate-type allergic reactions and inflammatory cytokine secretions in mast cells.[5]

Oxygen free radicals and reactive oxygen species have been suggested to play roles many diseases, such as cancer, atherosclerosis, liver disorders, inflammation, diabetes, and the aging process.[6,7] There have been many reports of different test methods for measuring antioxidant effects, including the ORAC test, which is one of the most popular and best standardized chemical antioxidant assays.[8-10] It is widely used for the evaluation and comparison of the antioxidant capacity in natural products and has also been successfully applied in bioavailability studies.[11] Reducing capacity and metal chelating capacity are also popular assays, frequently used in the determination of electron donating capacity of plant extracts or purified plant antioxidants (e.g., polyphenols).[12,13]

As part of our ongoing studies to find bioactive compounds from natural products, eight sesquiterpene compounds were isolated from the active fraction of *A. iwayomogi* (Fig. 1). In this paper, we report the isolation, structure determination, and antioxidant activity of these compounds using ORAC, reducing capacity, and metal chelating capacity assays.

Compound **1** was obtained as a colorless oil and its elemental composition was determined to be $C_{17}H_{20}O_7$ by HR-ESI-MS. The ^{1}H-NMR spectrum showed two olefinic proton signals at δ_H 6.15 (1H, d, J = 3.4 Hz) and 5.63 (1H, d, J = 3.4 Hz) and an acetyl signal at δ_H 2.06 (3H, s). The ^{13}C-NMR spectrum demonstrated the presence of 17 carbon signals. These were assigned to three methyl, two methylene, six methine, and six quaternary carbons by DEPT and HMQC spectra, composed of two olefinic carbon signals at δ_C 138.0 and 120.5, seven oxygenated carbon signals at δ_C 77.1, 58.1, 57.2, 70.1, 77.0, 71.5, and 69.8, and two carbonyl carbon signals at δ_C 170.0 and 170.5. These observations indicated that compound **1** was a guaianolide sesquiterpene lactone, similar to compound **4** except for four carbons at δ_C 77.1, 58.1, 57.2, and 69.8, which were also assigned to C-1, 2, 3, and 10 using HMBC experiments. Comparison of the chemical shift values in the ^{1}H- and ^{13}C-NMR spectra suggested that these four carbon signals should be two epoxy groups at δ_H 3.78 (d, J = 1.0 Hz) of δ_C 58.1 (C-2), δ_H 3.36 (d, J =1.0 Hz) of δ_C 57.2 (C-3), and δ_C 77.1 (C-1) and δ_C 69.8 (C-10), respectively, supported by those of ezoyomoginin.[14] Analysis of two-dimensional NMR data of compound **1** revealed its structure to be 1(10), 2(3)-diepoxy-4-hydroxy-8-acetoxy-guaia-11(13)-en-6,12-olide. From the coupling constant $J_{2\text{-}3}$ = 1.0 Hz, the epoxy group at the C2 and C3 positions must be a 2α(3α)-epoxy group. Furthermore, the relative stereochemistry of C4-C5-C6-C7-C8 was determined to be the same with compounds **4** and **5** on the basis of the coupling constants observed in the ^{1}H-NMR spectrum. These configurations of C-2 to C-8 and C-10 were confirmed by correlations between H-2/H-3, H-2/H-14, H-3/15, H-5/H-7, and H-6/H-8 in the NOESY spectrum. Thus, compound **1** was determined to be 1α(10α), 2α(3α)-diepoxy-4α-hydroxy-8α-acetoxy-guaia-11(13)-en-6α,12-olide, named as iwayoside B.

Compound **7** was obtained as a colorless oil and its elemental composition was determined to be $C_{21}H_{36}O_8$ by HR-ESI-MS. The ^{1}H-NMR spectrum revealed the presence of one β-glucopyranose unit from the anomeric proton signal at δ_H 4.32 (1H, d, J = 7.8 Hz), and the signals of three methyl groups at δ_H 1.14 (s), 1.04 (d, J = 7.2 Hz), and 1.01 (s), and an olefinic proton as a broad singlet at δ_H 5.31 of the aglycone moiety. The ^{13}C-NMR spectrum of compound **7** indicated the presence of one sugar unit, along with 15 carbon signals for the aglycone moiety, indicating a sesquiterpene skeleton. These signals were assigned to three methyl, five methylene, four methine, and three quaternary

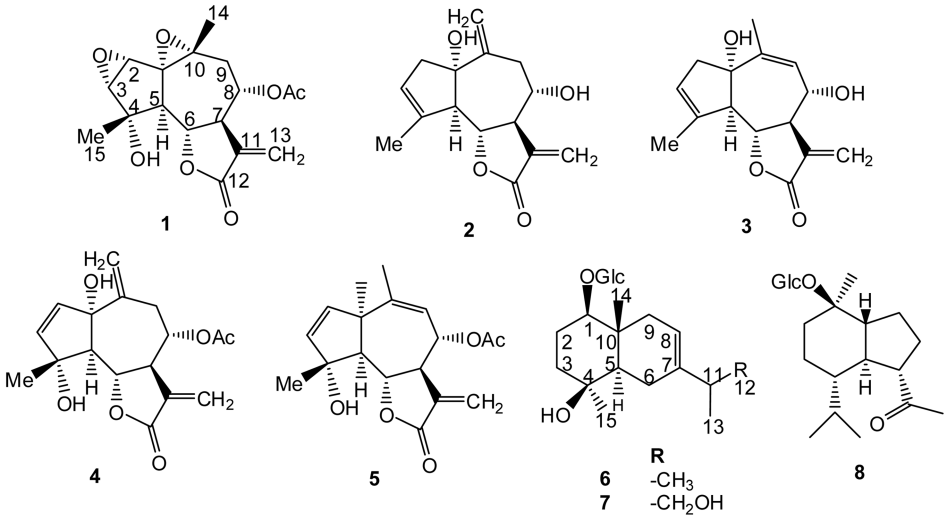

Figure 1. Structures of compounds **1-8**.

carbons by DEPT and HMQC spectra. These signals are similar to those of compound **6**, except for a carbon at δ_C 67.3 that was also assigned to C-12 using HMBC experiments. Lower field moving of this signal indicated that the methyl group was substituted by an oxymethylene group. Analysis of two-dimensional NMR data of compound **7** revealed its structure to be 7-eudesmene-1β,4β,12-triol 1-*O*-β-D-glucopyranose. COSY correlations between the oxymethylene protons [δ_H 3.58 (1H, m) and δ_H 3.40 (1H, m)] at C-12 and the H-11 proton [δ_H 2.22 (1H, m)] supported this structure. The sugar was expected to be attached to C-1, based on the HMBC correlations from proton H-1' at δ_H 4.32 to C-1 at δ_C 86.9. The peak of the acid hydrolysate of compound 7 was detected at t_R (min) 14.21 by GC analysis after treatment with trimethylsilylimidazole in pyridine. Retention times for authentic samples (Sigma), after being treated in the same manner, were 14.12 (D-glucose) and 14.25 (L-glucose). Co-injection of the hydrolysate of the sample with standard D-glucose gave a single peak. Thus, the structure of compound 7 was determined to be (11*S*)-7-eudesmene-1β,4β,12-triol 1-*O*-β-D-glucopyranose, named as iwayoside C.

Antioxidant capacities of each compound were determined using ORAC, electron donating capacity, and metal chelating capacity assays. On the basis of the chemical reactions involved, major antioxidant capacity assays can be roughly divided into hydrogen atom transfer reaction-based and single electron transfer reaction-based assays.[15] Reducing capacity assays involve an electron-transfer reaction, and the ORAC assay involves a hydrogen atom-transfer reaction. Although the DPPH assay is a popular method, the stable radical has no similarity to the highly reactive and transient peroxyl radicals; many antioxidants that react with peroxyl radicals may react slowly or may even be inert to DPPH, due to steric inaccessibility.[16] Thus, instead of the DPPH assay, the reducing capacity assay was used for the analysis of single electron transfer capacity.

All compounds tested, except compound **8**, showed significant peroxyl radical-scavenging capacity and reducing capacity (Fig. 2, 3), whereas only compound **3** showed a significant metal chelating capacity (Fig. 4). In the ORAC assay, compound **5** was found to have a little higher peroxyl radical-scavenging capacity than other compounds tested. When compared with quercetin as a positive control, the peroxyl radical-scavenging capacity of compound **5** was much weaker. The electron donating capacity of compounds **1-8** in the reducing capacity assay were similar to the pattern of their peroxyl radical-scavenging capacity. This suggests that the peroxyl radical-scavenging and reducing capacity of compounds **1-7**, due to hydrogen atom and single electron transfer, respectively, may be dependent on the chemical environment of the hydroxyl groups on the sesquiterpene skeleton. However, the metal chelating capacity of sesquiterpenes from *A. iwayomogi* was found only in compound **3**. The metal chelating capacity is also an important factor in

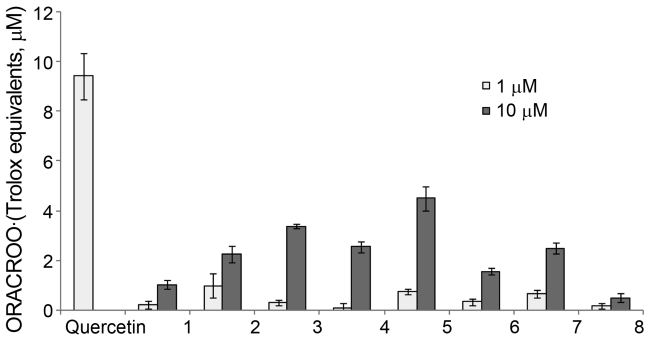

Figure 2. Peroxyl radical-scavenging activities of compounds **1-8** in ORAC system. The ORAC value is calculated by dividing the area under the sample curve by the area under the Trolox curve, with both areas being corrected by subtracting the area under the blank curve. One ORAC unit is assigned as the net area of protection provided by Trolox at a final concentration of 1 μM. The area under the curve from the sample is compared to the area under the curve for Trolox, and the antioxidative value is expressed in micromoles of Trolox equivalent per liter. The results represent the mean ± SD of values obtained from three measurements.

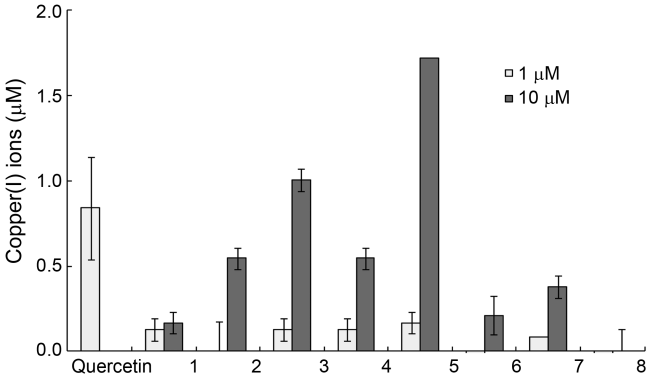

Figure 3. Reducing capacities of compounds **1-8**. The results represent the mean ± SD of values obtained from three measurements.

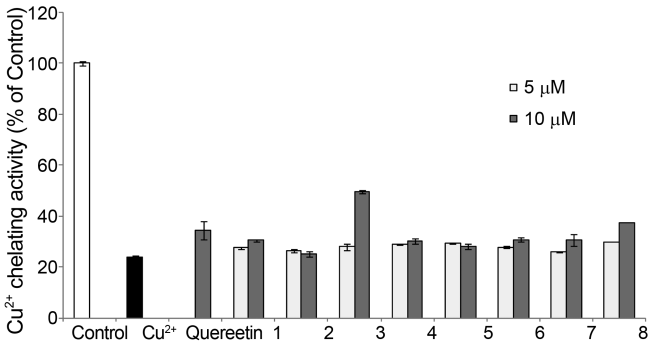

Figure 4. Metal chelating capacities of compounds **1-8**. The results represent the mean ± SD of values obtained from three measurements.

preventing the generation of hydroxyl radicals through the Fenton reaction between hydrogen peroxide and metal ions, such as iron and copper.[17] Thus, compound **3** may be useful as a chelating agent for sequestering free metal ions released due to various pathological symptoms.

In terms of structure-activity relationships in our experiment, the guaianolide and eudesmene-type sesquiterpenes showed the most significant peroxyl radical-scavenging and reducing capacity. Compounds **3** and **5**, both of which had the double bond in position C9-C10 of the guaianolide type, showed much higher activities than the other compounds. Assessing the metal-chelating capacity, only compound **3** of the guaianolide type showed good activity. Thus, 2,9-diene guaianolide-type sesquiterpene seems to be the key functional elements of the antioxidant properties.

Experimental Section

General Methods. Optical rotations were measured on a JASCO DIP-360 digital polarimeter. NMR spectra were recorded on a Bruker DRX 400 and 500 NMR spectrometers using TMS as an internal standard. HR-ESI-MS was carried out on a JMS-T100TD spectrometer (Tokyo, Japan). GC spectra were recorded on a Shidmazu-2010 spectrometer. Column chromatography was conducted using silica gel 60 (40-63 and 63-200 μm particle size) and RP-18 (40-63 μm particle size), which were both obtained from Merck.

Plant Materials. Aerial parts of *A. iwayomogi* (Compositae) were collected at Jeju island in June 2007 and taxonomically identified by one of us (Young Ho Kim). A voucher specimen (CNU07105) has been deposited at the herbarium of the College of Pharmacy, Chungnam National University, Korea.

Extraction and Isolation. The plants (3 kg) were extracted with 70% MeOH at room temperature for 1 day (3 × 10 L). The 70% MeOH extract (294 g) was concentrated under vacuum to give a gummy residue, which was then suspended in H_2O (3 L). This solution was extracted with EtOAc (3 × 3 L) to give 45 g of a EtOAc soluble fraction and 220 g of a H_2O soluble fraction. EtOAc soluble fraction (43 g) was chromatographed on a silica gel column eluted with a stepwise gradient of $CHCl_3$ and MeOH, to yield seven fractions (Fr.1-7). Fraction 3 was chromatographed on a silica gel column eluted with $CHCl_3$-MeOH stepwise gradient (20:1 → 10:1 → 5:1), to yield five subfractions (Fr.3A-3E). Compounds **2** (11 mg) and **5** (12 mg) were obtained from Fr.3B (0.2 g) using a reversed-phase (RP) C_{18} column eluted with MeOH-H_2O (1:1). Compound **1** (47 mg) were obtained from Fr.3D (0.3 g) using a RP C_{18} column eluted with MeOH-H_2O (1:1). Fraction 4 was chromatographed on a silica gel column eluted with $CHCl_3$-MeOH-H_2O (20:4:0.1), to yield four subfractions (Fr.4A-4D). Compound **3** (52 mg) was obtained from Fr.4C (0.25 g) using a RP C_{18} column eluted with MeOH-H_2O (1:1). Compound **4** (52 mg) was obtained from Fr.4D (0.3 g) using a RP C_{18} column eluted with MeOH-H_2O (4:5). The H_2O soluble fraction (215 g) was subjected to highly porous synthetic resin column chromatography using a stepwise gradient of H_2O-MeOH (1:0 → 3:1 → 1:1 → 1:3 → 0:1), to yield five fractions (Fr.8-12). Fraction 10 was chromatographed on a silica gel column eluted with $CHCl_3$-MeOH-H_2O stepwise gradient (20:1:0.1 → 10:1:0.1 → 5:1:0.1), to yield six subfractions (Fr.10A-10F). Compound **7** (9 mg) was obtained from Fr.10D (0.15 g) using a RP C_{18} column eluted with MeOH-H_2O (1:3). Fraction 11 was chromatographed on a silica gel column eluted with $CHCl_3$-MeOH-H_2O stepwise gradient (16:1:0.1 → 8:1:0.1 → 4:1:0.1), to yield 9 subfractions (Fr.11A-11I). Compounds **6** (5 mg) and **8** (9 mg) were obtained from Fr.11H (0.2 g) using a RP C_{18} column eluted with acetone-H_2O (1:2).

Iwayoside B (1): colorless oil; $[\alpha]_D^{20}$: +26.2 (*c* 0.1, MeOH); ^{1}H-NMR (CD_3OD, 500 MHz) and ^{13}C-NMR (CD_3OD, 125 MHz) see Table 1; ESI-MS *m/z*: 337 $[M + H]^+$; HR-ESI-MS *m/z*: 335.1125 $[M - H]^-$, (cacld for $C_{17}H_{19}O_7$, 335.1131).

Iwayoside C (7): colorless oil; $[\alpha]_D^{20}$: +41.3 (*c* 0.1, MeOH); ^{1}H-NMR (CD_3OD, 500 MHz) and ^{13}C-NMR (CD_3OD, 125 MHz) see Table 2; ESI-MS *m/z*: 417 $[M + H]^+$; HR-ESI-MS *m/z*: 417.2466 $[M + H]^+$, (cacld for $C_{21}H_{37}O_8$, 417.2488).

Oxygen Radical Absorbance Capacity (ORAC) Assay. The ORAC assay was performed using a Tecan GENios multi-functional plate reader (Salzburg, Austria) with fluore-

Table 1. NMR spectroscopic data (CD_3OD) for Compound **1**

Position	δ_C	δ_H (*J* in Hz)	HMBC	COSY
1	77.1			
2	58.1	3.78 (1H, d, 1.0)	1, 3, 4	
3	57.2	3.36 (1H, d, 1.0)	1, 2, 15	
4	70.1			
5	44.3	2.92 (1H, d, 11.0)	1, 4, 6, 7, 15	6
6	77.0	4.23 (1H, dd, 10.0, 11.0)	5, 8	
7	48.3	3.83 (1H, m)	5, 8, 11, 13	6, 8
8	71.5	5.19 (1H, ddd, 3.2, 7.2, 9.6)	10, 11, -OAc	9
9	43.7	2.25 (1H, dd, 7.2, 16.2) 1.75 (3.2, 16.2)	1, 7, 8, 10	
10	69.8			
11	138.0			
12	170.0			
13	120.5	6.15 (1H, d, 3.4) 5.63 (1H, d, 3.4)	7,11, 12	
14	24.9	0.97 (3H, s)	1, 9,10	
15	19.0	1.47 (3H, s)	3, 4, 5	
-OAc	170.5			
$-OCH_3$	19.8	2.06 (3H, s)	-OAc	

Table 2. NMR Spectroscopic Data (CD_3OD) for Compound **7**

Position	δ_C	δ_H (*J* in Hz)	HMBC	COSY
1	86.9	3.41 (1H, m)	1', 2, 3, 5, 10, 14	
2	23.9	1.70 (1H, m), 1.88 (1H, m)		1
3	40.3	1.75 (1H, m), 1.48 (1H, m)	1, 4, 5	2
4	71.5			
5	48.6	1.31 (1H, m)	1, 4, 6, 9, 10, 14, 15	
6	24.6	2.02 (1H, m), 2.11 (1H, m)	5, 7, 8	
7	139.3			
8	120.9	5.31 (1H, br. m)	6, 9, 10, 11	
9	42.1	2.15 (1H, m), 1.90 (1H, m)	5, 7, 10, 14	
10	38.4			
11	44.7	2.22 (1H, m)	6, 7, 8, 12, 13	
12	67.3	3.58 (1H, m), 3.40 (1H, m)	7, 11, 13	11
13	17.0	1.04 (3H, d, 7.2)	7, 11, 12	11
14	13.2	1.01 (3H, s)	1, 5, 9, 10	
15	30.0	1.14 (3H, s)	3, 4, 5	
1'	102.0	4.32 (1H, d, 7.8)	1, 5'	2'
2'	75.3	3.16 (1H, m)	1'	3'
3'	78.4	3.35 (1H, m)	2', 4'	
4'	72.1	3.27 (1H, m)	3', 5'	3'
5'	77.9	3.23 (1H, m)	4',	4'
6'	63.2	3.86 (2.1, 12.0), 3.56 (5.4, 12.0)	4', 5'	5'

scent filters (excitation wavelength: 485 nm, emission filter: 535 nm). In the final assay mixture, fluorescein (40 nM) was used as a target of free radical attack with AAPH (20 mM) as a peroxyl radical generator in peroxyl radical-scavenging capacity ($ORAC_{ROO\cdot}$) assay.[18]

Reducing Capacity. The reducing capacity of the samples tested measured the ability to reduce Cu^{2+} to Cu^{+} and was determined according to the method of Aruoma *et al.*[19]

Metal Chelating Activity. The metal chelating activity of each sample was assessed using the method of Argirova *et al.*,[20] with slight modifications.

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References

1. Lee, K. R.; Zee, O. P.; Kwak, J. H.; Kim, Y. S.; Park, H. K.; Koo, K. A.; Youn, H. J. *Kor. J. Pharmacogn.* **1993**, *24*, 289.
2. Ryu, J. H.; Ahn, H.; Kim, J. Y.; Kim, Y. K. *Phytother. Res.* **2003**, *17*, 485.
3. Ahn, H.; Kim, J. Y.; Kim, Y. K,; Ryu, J. H. *Arch. Pharm. Res.* **2003**, *26*, 301.
4. Kim, A. R.; Zou, Y. N.; Park, T. H.; Shim, K. H.; Kim, M. S.; Kim, N. D.; Kim, J. D.; Bae, S. J.; Choi, J. S.; Chung, H. Y. *Phytother. Res.* **2004**, *18*, 1.
5. Shin, T. Y.; Park, J. S.; Kim, S. H. *Immunopharm. Immunot.* **2006**, *28*, 421.
6. Halliwell, B. *Lancet* **1994**, *334*, 721.
7. Fukujawa, K.; Takaishi, Y. J. *J. Act. Oxyg, Free Rad.* **1990**, *1*, 55.
8. Ou, B.; Hampsch-Woodill, M.; Prior, R. L. *J. Agric. Food Chem*. **2001**, *49*, 4619.
9. Prior, R. L.; Hoang, H.; Gu, L.; Wu, X.; Bacchiocca, M.; Howard, L.; Hampsch-Woodill, M.; Huang, D.; Ou, B.; Jacob, R. *J. Agric. Food Chem.* **2003**, *51*, 3273.
10. Huang, D.; Ou, B.; Prior, R. L. *J. Agric. Food Chem.* **2005**, *53*, 1841.
11. Prior, R. L.; Gu, L.; Wu, X.; Jacob, R. A.; Sotoudeh, G.; Kader, A. A.; Cook, R. A. *J. Am. Coll. Nutr.* **2007**, *26*, 170.
12. Park, Y.-S.; Jung, S.-T.; Kang, S.-G.; Heo, B. G.; Lee, S.-H.; Toledo, F.; Arancibia-Avila, P.; Drzewiecki, J.; Gorinstein, S. *J. Food Biochem.* **2009**, *33*, 674.
13. Sun, J.; Yao, J. Y.; Huang, S. X.; Long, X.; Wang, J. B.; Garcia-Garcia, E. *Food Chem.* **2009**, *117*, 276.
14. Nagaki, M.; Matsueda, S. *Phytochemistry* **1989**, *28*, 2731.
15. Huang, D. J.; Ou, B. X.; Ronald L. Prior. *J. Agric. Food Chem.* **2005**, *53*, 1841.
16. Prior, R. L.; Wu, X. L.; Schaich, K. *J. Agric. Food Chem.* **2005**, *53*, 4290.
17. Aruoma, O. *Methods Enzymol.* **1994**, *233*, 57.
18. Kurihara, H.; Fukami, H.; Asami, S.; Totoda, Y.; Nakai, M.; Shibata, H.; Yao, X. S. *Biol. Pharm. Bull.* **2004**, *27*, 1093.
19. Aruoma, O. I.; Murcia, A.; Butler, J.; Halliwell, B. *J. Agric. Food Chem.* **1993**, *41*, 1880.
20. Argirova, A. D. Ortwerth, B. J. *Arch. Biochem. Biophys.* **2003**, *420*, 176.